



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Publication number:

0 411 925 A3

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 90308469.7

(51) Int. Cl.⁵: C08H 1/06

(22) Date of filing: 01.08.90

(30) Priority: 02.08.89 US 388003
30.07.90 US 557639

(43) Date of publication of application:
06.02.91 Bulletin 91/06

(64) Designated Contracting States:
CH DE ES FR GB IT LI

(66) Date of deferred publication of the search report:
14.08.91 Bulletin 91/33

(71) Applicant: UNIVERSITY OF NORTH CAROLINA
AT CHAPEL HILL

Chapel Hill, North Carolina 27514(US)

(72) Inventor: Mechanic, Gerald L.
2313 Honeysuckle Road Chapel Hill
North Carolina 27514(US)

(74) Representative: Brown, John David et al
FORRESTER & BOEHMERT
Widenmayerstrasse 4/I
W-8000 München 22(DE)

(54) Process for cross-linking collagenous materials and resulting product.

(57) The present invention relates to a process for cross-linking a proteinaceous material. The process comprises:

i) soaking the material to be cross-linked in an aqueous solution of high osmolality; ii) incubating the material in an aqueous buffer including an amount of a photooxidative catalyst sufficient to catalyze photooxidation of the material; and iii) irradiating the material and the catalyst of step (i) with light that includes a range of wavelengths selectively absorbed by the catalyst. Irradiation is effected under conditions such that cross-linking of the material occurs. In a further embodiment, the present invention relates to a cross-linked product produced by the above-described method.

EP 0 411 925 A3



European
Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 30 8469

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.6)
P,A	US-A-3 152 978 (ELOIS KUNTZ) - - -		C 08 H 1/06 A 61 L 27/00
A	WPI / DERWENT, accession no. 71-12949S [07], Derwent Publications Ltd, London, GB; & JP-B-46 006 382 (JAPAN LEATHER CO.,LTD) - - -		
P,Y	PATENT ABSTRACTS OF JAPAN, vol. 14, no. 275 (C-278)[4218], 14th June 1990; & JP-A-2-083 395 (TEIJIN) 23-03-1990 - - -	1-16	
Y	CHEMICAL ABSTRACTS, vol. 72, no. 9, 2nd March 1970, page 24, abstract no. 38990x, Columbus, Ohio, US; C. GOWRI et al.: "Photoxidation of collagen in the presence of methylene blue", & LEATHER SCI. (MADRAS) 1969, 16(8), 297-300 * Whole abstract * - - -	1-16	
Y	DAS LEDER, vol. 19, no. 1, January 1968, pages 1-8, Darmstadt, DE; H. ZAHN et al.: "Reaktivbarbstoffe als Gerbstoffe für Kollagenfolie" * Pages 6,7 * - - - - -	1-16	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 08 H C 09 H A 61 L
The present search report has been drawn up for all claims			
Place of search		Date of completion of search	Examiner
The Hague		23 May 91	LENSEN H.W.M.
CATEGORY OF CITED DOCUMENTS X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons &: member of the same patent family, corresponding document			

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61L 27/36, 27/26	A1	(11) International Publication Number: WO 00/15274 (43) International Publication Date: 23 March 2000 (23.03.00)
(21) International Application Number: PCT/GB99/03013 (22) International Filing Date: 10 September 1999 (10.09.99) (30) Priority Data: 9819882.3 11 September 1998 (11.09.98) GB (71) Applicant (for all designated States except US): TISSUE SCIENCE LABORATORIES LIMITED [GB/GB]; Greyholme House, 49 Victoria Road, Aldershot, Hampshire GU11 1SJ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): OLIVER, Roy, Frederick [GB/GB]; Priorwell House, Gauldry, Fife DD6 8SE (GB). GRANT, Roy, Arthur [GB/GB]; 15 Pine Park Mansions, 1-3 Wilderton Road, Branksome Park, Poole, Dorset BH13 6EB (GB). (74) Agent: GALLAFENT & CO.; 9 Staple Inn, London WC1V 7QH (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: COLLAGENOUS TISSUE COMPOSITIONS (57) Abstract <p>Implant compositions are disclosed consisting of a biocompatible carrier medium such as a saline or dextran solution and particles of collagenous material dispersed therein. The collagenous material is derived from tissue which has been milled to provide fragments of collagen fibres which preserve the architecture of the original fibres and their molecular structure. The collagenous material is also substantially free of non-fibrous tissue proteins, glycoproteins, cellular elements and lipids or lipid residues, and is non-cytotoxic. By suitable choice of particle size and concentration, the composition may be presented in injectable form or as a paste. The compositions are suitable for application in cosmetic and reconstruction surgery.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

- 1 -

COLLAGENOUS TISSUE COMPOSITIONS

This invention relates to collagenous tissue compositions.

5

In recent years, much attention has been given to the development of compositions and preparations for wound treatment and for use in general and plastic surgery, in particular for the improved restoration of surgically
10 induced wounds or for the correction of physiological malfunction as, for example, of the urethral sphincter in cases of urinary incontinence.

Much attention has been focussed on the provision of
15 materials based on collagen, either of human or animal origin. In particular, considerable attention has been directed to developing preparations and materials based on animal tissues which are treated to provide compatibility, i.e. to avoid rejection of the tissues
20 when used on humans.

Earlier work by the inventors of the present application is reflected in United States Patent Specification 5397353 and EP-A-182842 which disclose methods of

- 2 -

preparing collagenous materials, preferably in sheet form, and which are suitable for transplantation. The treatment is designed to produce a collagenous material which is non-antigenic so that it is not rejected and which is non-resorbable so that it forms a permanent transplant. In particular, the material described in these specifications retains the natural structure and original architecture of the natural tissue; the molecular ultrastructure of the collagen is retained.

10 These materials have proved highly satisfactory in practice and, in particular, have shown themselves to be capable of being re-vascularised once implanted while, at the same time, being resistant to calcification. They are particularly useful in ear, nose and throat,

15 orthopaedic, gynaecological and urological procedures and a range of hernia repairs including parastomal incisional and inguinal hernias.

The compositions described in United States Patent Specification 5397353, however, are disclosed as large scale structures, for example 0.75 mm thick and usually presented as sheets varying in size from 25 cm² to 50 cm². This is useful for specific implant use, e.g. during restorative surgery, but is not always suited for

25 use generally to build up soft tissues.

In cosmetic and reconstructive surgery, e.g. for the repair of small acne scars and for elevating and smoothing wrinkles, it is often desirable to use

30 material in another form for tissue implantation or so-called augmentation which can be injected or otherwise introduced into the desired site.

Various so-called injectable implant materials have been developed for such purposes. United States Patent Specifications 5523291, 5676698 and 5705488 disclose

35

- 3 -

injectable implant compositions for soft tissue augmentation comprising elastin and collagen and a biocompatible carrier, or flexible pouches containing such a material. The difficulty with such materials as
5 are disclosed in these United States specifications, however, is that there is a tendency to resorption and this can mean that the implant is effective only for a limited time. Additionally, such materials do not encourage vascularisation, i.e. they do not integrate
10 well into the surrounding healthy tissue following implantation.

Furthermore, in wound surgery, e.g. for repairing bullet wounds or injuries caused by machinery or vehicle
15 accidents and indeed following incisional injury, there is often a problem in that tissue is lost from the wound area. This leads to the development of scars, which may be hyperplastic and disfiguring and lead to impaired body function.

20 Scars arise from the biological response of adult connective tissue to injury. Unlike foetal tissues which respond to incision or injury by regenerating new dermis to replace the lost/damaged tissue (i.e. bridging
25 the defect with dermal collagen fibres with normal dermal collagen architecture) after birth, equivalent wounds are repaired rather than regenerated and the wound becomes filled with scar tissue. Thus the bridging tissue after birth does not replicate the
30 original normal dermal architecture. During the repair process, fibroblasts (the cells which permeate all connective tissues and which synthesise the extra-cellular matrix including structural collagen) and small blood vessels migrate into the wound space to form
35 highly cellular granulation tissue which transforms into the dense irregularly organised collagen mass described

- 4 -

as scar tissue.

One solution to this particular tissue loss problem has been to apply three-dimensional collagen gels within the lost tissue area of the wound which subsequently acts as a matrix network for the growth of so-called histiotypic skin. The collagen used to form this particular gel is completely water-soluble and when it is applied, it is invaded with fibroblasts and small blood vessels, water is extruded and a fragile gel is formed in which the collagen molecule is polymerised to form collagen fibrils. Although reasonably successful in rebuilding the lost tissue in the area around the original wound, the initial three-dimensional matrix formed from the collagen gel does not replicate the normal matrix architecture of the body's natural tissue and, as such, the gel has no inherent stability. This inherent instability leads to the gel being rapidly re-absorbed by the body and replaced with scar-like tissue.

Other recent proposals to overcome the problem of scar tissue formation have involved the extremely difficult (and very expensive) use of monoclonal antibodies to suppress the action of growth factors such as transforming growth factor (TGF - β).

We have now surprisingly found that the favourable properties, including resistance to resorption, resistance to calcification, granulation and the ability to become recellularized and revascularised, which characterise the large scale structures disclosed in Specification 5397353, are capable of being retained if the collagen material is presented in mouldable form at the fibre fragment level of organisation, where it can be used as a wound filler, or in injectable form for use in cosmetic and reconstructive surgery.

- 5 -

According broadly to the present invention there is provided an implant composition which comprises a biocompatible carrier medium having dispersed therein particles of collagenous material, where the particles
5 comprise fragments of collagenous fibres and are thus sufficiently large to preserve the original architecture and molecular structure of the natural tissue material from which they are derived, and wherein the collagenous material is substantially free of non-fibrous tissue
10 proteins, glycoproteins, cellular elements and lipids or lipid residues, and which is non-cytotoxic. Preferably, the material is free or substantially free of antigenic polysaccharides and mucopolysaccharides. The biocompatible medium may be, for example, a saline or
15 dextran or hyaluronic acid solution.

Such compositions may vary widely in consistency. For example, if the particle size and concentration in the biocompatible medium is such as to produce a fairly
20 liquid suspension, this can be injectable provided the particles are not too large. More concentrated thicker consistency compositions may be used as pasty wound filling compositions.

25 Such materials may be prepared from collagenous materials of human or animal origin, the preferred starting material being pig dermis, by methods as disclosed in Specification 5397353 or analogously thereto. Depending on the starting material, the
30 composition may contain a proportion of elastin. It is then possible, provided care is taken, to reduce the material from large pieces to small particles which can then be formulated into a sterile injectable composition or a sterile wound filling paste.

35

In order to produce a collagen paste with appropriate

- 6 -

density and rheological properties (flow rate and an ability to retain shape after moulding), a suspension of collagenous particles in a suitable carrier can be prepared to form a controllable concentration of the composition.

Care must however be taken to ensure that the size reduction of the starting material is not accompanied by degradation of the molecular structure of the original material. The preferred method of providing particles of an appropriate size is by grinding or milling and this is preferably carried out in a ball or hammer mill which may be cooled to an appropriate temperature. Milling may be carried out in dry form (less than 10% moisture content) or in frozen hydrated form (20 - 80% moisture content).

Collagen which has been milled in a frozen hydrated state may be dehydrated by acetone extraction, freeze drying or in a current of air. The dry collagen powder may be suspended in an essentially non-aqueous, non-toxic, bio-compatible medium, such as for example, glycerol prior to injection.

An anaesthetic as for example, lignocaine may be incorporated into the composition.

The collagenous material may be, if desired, crosslinked, e.g. using a diisocyanate, in order to make it resistant to collagenolytic enzymes and thus render it substantially non-resorbable.

The preferred method of rendering the compositions sterile is by gamma irradiation.

- 7 -

The preferred particle size of the particles of collagenous material in the injectable compositions according to the present invention is from 50 to 500 microns. The particle size distribution may vary but
5 preferably at least 50% of the particles are within \pm 35% of the average particle size. The concentration of solids in the injectable composition is preferably in the range of 10 to 70% (w/v). In contrast, in the pasty wound filling compositions, the concentration of solids
10 is generally up to 80%.

The efficacy of the compositions of the invention can be seen in vitro. It has been observed that when dispersed collagen fibre fragments (milled collagen) are seeded
15 with human or rodent fibroblasts in tissue culture, the fibroblasts attach to the collagen fragments and aggregate them to form dense tissue like discs which are easily manipulable.

20 Furthermore, when injected in vivo, milled collagen is rapidly invaded by fibroblasts and small blood vessels (much more rapidly than collagen sheets) to form a new tissue in which the collagen fibre fragments are organised into intermeshing collagen fibres similar to
25 normal dermal collagen architecture, i.e. are not resorbed and do not form scar tissue.

The injectable compositions can be used in a variety of clinical situations. For example, to control urinary
30 incontinence and more specifically in intrinsic sphincter deficiency, by peri-urethral injection to reduce lumen aperture. Cosmetic applications include the use of injection of collagenous suspensions following eyebrow uplift, for lip augmentation and to
35 rectify facial defects, frown lines and acne scars. As another example, in arthritic joints, there is often a

- 8 -

marked loss and damage of the smooth cartilage layer which consists of chondrocytes supported by a fibrous collagen matrix. There is evidence that under the inflammatory conditions in arthritic joints that

5 collagenase is produced which destroys the collagen matrix of the cartilage layer. If a collagenous suspension according to the invention is injected into the joint, it may assist in producing a collagenase resistant matrix to support chondrocytes and so repair

10 the damage.

An alternative clinical scenario is where it is necessary to treat a large area of skin, for example, the back of the hand or neck in elderly patients where

15 the skin has become very thin. A multi-point injection system may be employed for this purpose. Such a system may combine a number of needles mounted in a hollow block of metal or plastics material, the inlet of which is fed with collagenous suspension with a syringe,

20 metering pump, piston peristaltic pump or any other suitable device.

The collagenous compositions of the invention may also be used for the purpose of suppressing scar formation in

25 surgical wounds, the milled collagenous material again serving to introduce fibre-structured fragments into the wound space immediately during or after closing the wound by suture or tape. Although totally against convention, such a procedure has been shown to be

30 extremely beneficial. The introduction of the collagenous material fragments into newly-formed wounds, e.g. incisional spaces, provides an anatomically "thin" matrix of collagen-rich sites for the fibroblasts and small blood vessels to migrate in to from the wound

35 edges. This has a profound influence on the behaviour of the fibroblasts as within such a collagen-rich

- 9 -

environment within the wound space, they do not receive the signals to produce granulation tissue and synthesise excess new collagen. In other words scar formation is largely suppressed. This simple "mechanical" approach differs from the prior art, in particular the use of monoclonal antibodies as it is far simpler to apply and far cheaper.

10 Use of milled collagen by injection through fine needles is somewhat limited because of the mode of introduction of collagenous material to the site where it is needed. However, the thicker consistency compositions, which allow the use of a wider spectrum of collagenous
15 material fragment sizes, can be used in a variety of situations where an injectable material would not be suitable. Thus in the treatment of more extensive or severe wounds, in order to replace lost tissue and to greatly reduce the formation of scar tissue, collagen
20 fibre fragments may be introduced as a pasty composition into the wound space before applying an appropriate dressing or closure by suture or tape. For example, the composition may be used for immediate reconstruction following breast lumpectomy. For skin-loss defects,
25 including those following traumatic chemical or burn injury, or those presented by leg ulcers, the pasty composition may be used to replace lost dermis with appropriate cover and dressing.

30 The following examples will serve to illustrate the invention:

Example 1

35 Under sterile conditions, samples of porcine dermal collagen were cut into small pieces (1 to 3 mm³) and

- 10 -

dehydrated using several changes of 100% ethanol and anhydrous acetone. Using a ball mill, the dried collagen pieces were ground and sieved to produce a fine white powder. The sieved powdered collagen was
5 rehydrated in sterile phosphate buffered saline to produce a collagen suspension concentration of 60 to 70% (w/v).

Example 2

10

Small pieces of blotted porcine collagen were frozen in liquid nitrogen and ground in a cryogenic mill. The ground collagen fragments were suspended in sterile phosphate buffered saline to produce a collagen
15 suspension concentration of 60 to 70% (w/v).

Example 3

To directly examine cell/collagen biointeraction, sieved
20 powdered porcine dermal collagen was rehydrated in complete mammalian cell culture medium to produce a collagen suspension concentration of 70% (w/v) and seeded with either primary human foreskin fibroblasts or primary rat skin dermal fibroblasts.
25 Collagen/fibroblast samples were aliquoted into costar wells and incubated at 37°C, 5 to 7% (w/v) CO₂ saturated humidity. As studied over a 21 day incubation period, both human and rat fibroblasts proliferated and migrated into and adhered to the porcine collagen fragments which
30 they assembled into densely packed clumps or discs.

Example 4

To examine in vivo performance collagen suspensions were
35 injected (0.2 ml/injection) through a 21 gauge needle intracutaneously into dorsal sites in isogenic PVG/Ola

- 11 -

rats. Sequential biopsies up to 12 month post injection showed the persisting macroscopic presence of injected collagen as subdermally located white discs with no overt signs of loss of injected collagen mass nor of adverse host reactions. Early biopsies showed that the injected collagen remains in situ and within 9 days is fully invaded with fibroblasts and small blood_vessels. Subsequent histology showed that the collagen fibre fragments are organised into intermeshing collagen fibres to produce a tissue with an architecture resembling normal dermal collagen.

Example 5

Under sterile conditions, samples of porcine dermal collagen produced in accordance with the process described in US-A-5397353 were cut into small pieces (1 to 3 mm³), frozen in liquid nitrogen and ground in a cryogenic mill. The ground collagen fragments were suspended in sterile phosphate buffered saline to produce a pasty composition with a solids content of 80%w/v.

Example 6

Pockets were made in the skin of the pinnae of PG/Ola rats, the collagen paste composition inserted with a spatula and the wounds closed and secured with a spray dressing. Sites of collagen insertion were biopsied at monthly intervals for histological examination. Over a period of 6 months, the collagen implants which persisted as raised skin bumps, became incorporated into surrounding host tissues and no adverse effects were found.

Example 7

- 12 -

1 ml of the collagen paste was injected by "trocar" or large bore needle subdermally in the dorsum of PVG/Ola rats. This "soft tissue filler" persisted with no adverse host reactions over a period of six months.

5

Example 8

Full-thickness incisional skin wounds were made in the dorsum of PVG/Ola rats. The wounds were closed using interrupted sutures and a suspension of collagen composition was injected into the wounds until it extruded above the wound surface. Wounds were biopsied at 6, 8, 10 and 14 days for histological examination which revealed evidence of incisional healing in the absence of observable scar tissue.

15

Example 9

The collagen paste composition, with or without prior seeding with isogenic fibroblasts in culture, was used to fill 1 x 1cm full-thickness excised skin wounds in PVG/Ola rats and covered with a semi-permeable membrane (Opsite - REGISTERED TRADE MARK) as a primary dressing. Subsequent observation and histology revealed that the implanted collagen composition becomes covered by migrating epithelium from the wound margins within 28 days and acts as an effective and persisting dermal replacement.

25

30

- 13 -

CLAIMS

1. An implant composition which comprises a biocompatible carrier medium having dispersed therein
5 particles of collagenous material where the particles are sufficiently large to preserve the original fibre and molecular structure of the natural tissue material from which they are derived and wherein the collagenous material is substantially free of non-fibrous tissue
10 proteins, glycoproteins, cellular elements and lipids or lipid residues and which is non-cytotoxic.
2. A composition according to Claim 1 wherein the collagenous material is free or substantially free of
15 antigenic polysaccharides and mucopolysaccharides.
3. A composition according to Claim 1 or 2 wherein the biocompatible medium is saline, dextran solution, or glycerol or a non-toxic antigenic viscous
20 polysaccharide.
4. A composition according to any one of Claims 1 to 3 wherein the collagenous material contains a proportion of elastin.
25
5. A composition according to any one of Claims 1 to 4 wherein the collagenous material is cross-linked.
6. A method according to any one of Claims 1 to 5
30 wherein the particle size of the particles of collagenous material is within the range of 50 to 500 microns.
7. A composition according to any one of the preceding
35 Claims wherein the concentration of solids is 10 to 70 percent by weight and the consistency of the composition

- 14 -

is such as to enable it to administered by injection.

8. A composition according to any one of the preceeding
claims wherein the composition is of a pasty
5 consistency.

9. An implant composition substantially as
hereinbefore described with reference to the foregoing
specific examples.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/03013

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61L27/36 A61L27/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 251 695 A (COLLAGEN CORP) 7 January 1988 (1988-01-07) claims; examples 1-4 ---	1-9
X	US 5 256 140 A (FALLICK HARRY) 26 October 1993 (1993-10-26) claims ---	1-9
X	US 4 582 640 A (SMESTAD THOMAS L ET AL) 15 April 1986 (1986-04-15) claims ---	1-9
X	US 4 837 285 A (BERG RICHARD A ET AL) 6 June 1989 (1989-06-06) claims; examples ---	1-9
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

10 December 1999

Date of mailing of the international search report

20/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

ESPINOSA, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/03013

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 13755 A (COLETICA) 22 July 1993 (1993-07-22) claims ---	1-9
A	EP 0 697 218 A (COLLAGEN CORP) 21 February 1996 (1996-02-21) claims; examples 1-10 ---	1-9
A	EP 0 083 868 A (COLLAGEN CORP) 20 July 1983 (1983-07-20) claims ---	1-9
A	US 5 523 291 A (JANZEN ERNST ET AL) 4 June 1996 (1996-06-04) cited in the application claims; example ---	1-9
A	US 5 705 488 A (JANZEN ERNST ET AL) 6 January 1998 (1998-01-06) cited in the application claims -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/03013

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0251695	A	07-01-1988	US 4803075 A	07-02-1989
			AU 7467187 A	07-01-1988
			JP 1899011 C	23-01-1995
			JP 6022581 B	30-03-1994
			JP 63119772 A	24-05-1988
US 5256140	A	26-10-1993	NONE	
US 4582640	A	15-04-1986	AT 27821 T	15-07-1987
			CA 1224210 A	14-07-1987
			EP 0089145 A	21-09-1983
			JP 1036840 B	02-08-1989
			JP 1554411 C	23-04-1990
			JP 58170796 A	07-10-1983
			JP 1085661 A	30-03-1989
			JP 1791992 C	14-10-1993
			JP 4078311 B	10-12-1992
US 4837285	A	06-06-1989	US 4970298 A	13-11-1990
			AU 4210585 A	01-11-1985
			BR 8506206 A	15-04-1986
			CA 1295796 A	18-02-1992
			DE 3585069 A	13-02-1992
			DK 547485 A	24-01-1986
			EP 0177573 A	16-04-1986
			ES 541629 A	01-04-1986
			FI 854692 A	27-11-1985
			IT 1182725 B	05-10-1987
			JP 8011121 B	07-02-1996
			JP 61502129 T	25-09-1986
			MX 163953 B	03-07-1992
			NO 854723 A	26-11-1985
			WO 8504413 A	10-10-1985
			US 4841962 A	27-06-1989
			US 4925924 A	15-05-1990
			US 4937323 A	26-06-1990
			US 4703108 A	27-10-1990
WO 9313755	A	22-07-1993	FR 2686250 A	23-07-1993
			AT 136773 T	15-05-1996
			DE 69302262 D	23-05-1996
			DE 69302262 T	19-09-1996
			EP 0621776 A	02-11-1994
			ES 2090969 T	16-10-1996
			JP 7503001 T	30-03-1995
			US 5658593 A	19-08-1997
EP 0697218	A	21-02-1996	US 5550187 A	27-08-1996
			US 5643464 A	01-07-1997
			US 5527856 A	18-06-1997
EP 0083868	A	20-07-1983	US 4424208 A	03-01-1984
			CA 1199580 A	21-01-1986
			JP 1329859 C	30-07-1986
			JP 58121958 A	20-07-1983
			JP 60054288 B	29-11-1985
US 5523291	A	04-06-1996	AU 675879 B	20-02-1997

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/03013

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5523291 A		AU 7685794 A	27-03-1995
		BR 9407420 A	12-11-1996
		CA 2170754 A	16-03-1995
		CN 1133562 A	16-10-1996
		EP 0717634 A	26-06-1996
		JP 9502372 T	11-03-1997
		WO 9507095 A	16-03-1995
		US 5705488 A	06-01-1998
US 5705488 A	06-01-1998	US 5523291 A	04-06-1996
		AU 675879 B	20-02-1997
		AU 7685794 A	27-03-1995
		BR 9407420 A	12-11-1996
		CA 2170754 A	16-03-1995
		CN 1133562 A	16-10-1996
		EP 0717634 A	26-06-1996
		JP 9502372 T	11-03-1997
		WO 9507095 A	16-03-1995